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**Immuno-oncologic approaches: CAR-T cells and checkpoint inhibitors**

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**Abstract (words-max 250: 250)**

Advances in understanding the myeloma biology have shown that disease progression is not only the consequence of intrinsic tumor changes but also of interactions between the tumor and the microenvironment in which the cancer grows. Immune system is an important component of the tumor microenvironment in myeloma, and acting on immune system is an appealing new treatment strategy. There are 2 ways to act towards immune cells and boost anti-tumor immunity: 1) to increase antitumor activity (acting on T and NK cytotoxic cells); and 2) to reduce immunosuppression (acting on myeloid-derived stem-cells and T regulatory cells). Checkpoint inhibitors and adoptive cell therapy (ACT) are two of the main actors, together with monoclonal antibodies and immunomodulatory agents, in the immune-oncologic approach. The aim of checkpoint inhibitors is to release the brakes that block the action of the immune system against the tumor. Anti-programmer death-1 (PD1) and PD1-Ligand, as well as anti-CTLA4 and KIR are currently under evaluation, as single agents or in combination, with so far the best results achieved with combination of anti-PD1 and immunomodulatory agents. The aim of ACT is to create an immune effector specific against the tumor. Preliminary results on chimeric antigen receptor (CAR) T cells, first against CD19, and more recently against B cell maturation antigen, have shown to induce durable responses in heavily pretreated patients. This review will focus on the most recent clinical results available on the use of checkpoint inhibitors and CAR-T cells in myeloma, in the context of the new immune-oncologic approach.

**Key words:** check-point inhibitors, CAR-T cells, immune-oncology

Text word count: 3959

## **Introduction**

There have been significant advances in the understanding of the biology of multiple myeloma (MM) in recent years. In the classical view of the pathogenesis of MM, an initiating hit is necessary to immortalize a myeloma-propagating cell. This cell then acquires additional genetic hits over time, mediated by translocation, loss of heterozygosity, gene amplification, mutation, or epigenetic changes, which further deregulate the behavior of the MM-propagating cell, leading step by step to the well-known MM features.<sup>1</sup> Many of the genes and pathways mediating this transformation process have now been characterized. Nevertheless, disease progression is not only the consequence of intrinsic tumor changes. Interactions between the tumor and the microenvironment in which the cancer grows, play an essential role,<sup>2</sup> and the focus of studies has shifted from the disease itself alone to the disease in the context of the microenvironment where the tumor grows. Immune system is an important component of the tumor microenvironment in MM, as well as in many other cancers, and is the focus of the immune-oncology approach.

## **Rationale for immune-oncology**

The immune system can potentially recognize and reject the tumor. Tumor cells can express aberrant antigens i.e. molecules expressed in tumor cells, but not in normal cells. These can be normal cellular proteins that are abnormally expressed as a result of genetic mutations, quantitative differences in expression, or differences in posttranslational modifications.<sup>3</sup> In tumor types that have a well-documented viral origin, viral proteins can also serve as tumor antigens.<sup>4,5</sup> Tumor antigens are first recognized by the innate immune system subsets, among which natural killer (NK) cells have the capability to kill tumor cells. Macrophages and dendritic cells then uptake and process fragments from these destructed cells, secrete inflammatory cytokines and present tumor cell-derived molecules to T and B cells. Activation of T- and B-cells leads to the expansion of tumor-specific cell clones and antibodies. Moreover, adaptive immunity subsets produce additional cytokines that further promote activation of innate immunity. The final goal of the adaptive immune system is to eliminate the remaining tumor cells and to generate immune memory against specific tumor components, thus preventing tumor recurrence.<sup>6</sup>

This process can result in different outcomes. A highly immunogenic tumor in a highly immunocompetent individual can eliminate the arising tumor. In a less immunocompetent individual and/or in case of less

immunogenic tumor, there can be an incomplete elimination leading to the survival of some cancer cells that nevertheless remain under immunosurveillance. This phase could be at some point disturbed by changes in the tumor that allow it to avoid immunosurveillance, or changes in the immune system that weaken its capacity to keep the tumor under control, leading to tumor escape.<sup>7</sup> The escape phase of immunosurveillance is characterized by an increase in immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC). Immunosuppressive cytokines derived from Tregs, MDSC and tumor cells themselves can hamper the effector function of T cells.<sup>8–10</sup>

MM is characterized by a profound immune dysfunction affecting both the innate and adaptive immune system.<sup>11</sup> Antigen cross-presentation is the dominant mechanism of tumor antigen priming<sup>12</sup> and as a consequence, the functional status of antigen presenting cells (APCs) is crucial. Dendritic cells from MM patients are functionally impaired and express/produce lower levels of critical molecules that can initiate the immune response (such as interleukin 12 (IL-12), HLA-DR, CD40, CD86, and CD80).<sup>13,14</sup> Regulatory T cells may exert immunosuppression by several mechanisms, including production of anti-inflammatory cytokines (IL-10 and TGF $\beta$ ), and reduction in IL-2.<sup>15</sup> MDSCs inhibit T cells by producing arginase-1, reactive oxygen species, and nitric oxide.<sup>16</sup> MM cells themselves play an important role in maintaining immunosuppression. As an example, they can produce TGF $\beta$  and express PDL-1, both leading to T cells inhibition. Taken together, all these mechanisms suggest a complex interaction between MM cells and the immune system, with several possible targets for the immune-oncologic approach.

### **Immune-oncologic approach in MM**

Immune therapy can be directed against the tumor itself or towards immune cells. There are 2 strategies to act towards immune cells and boost anti-tumor immunity: one consists in increasing antitumor activity (acting on T and NK cytotoxic cells), the other one in reducing immunosuppression (acting on MDSC and Tregs). Treatment approaches include monoclonal antibodies (mAbs) targeting surface molecules present on MM cells, mAbs targeting checkpoint inhibitors on immune cells/tumor cells, immunomodulation, vaccines and adoptive cell therapy (ACT). Several targets, however, are expressed on both MM cells and immune cells, thus leading to complex mechanism of actions.

The review focuses on checkpoint inhibitors and chimeric antigen receptor (CAR)-T cells based ACT (*Figure 1*). To contextualize the role of checkpoint inhibitors and ACT in the immune-oncology approach,

we will provide a brief summary of the main mechanisms by which mAbs targeting surface molecules present on the MM cells, immunomodulation and vaccines act on the immune system .

Elotuzumab is a mAb targeting SLAMF7 (also known as CS-1), present on both MM cells and NK cells. It causes MM cell death via a dual mechanism of action. The Fab portion of Elotuzumab binds SLAM F7 on MM cells, the Fc portion binds CD16 on NK. This interaction triggers NK activation, release of cytotoxic granules and MM cell killing.<sup>17,18</sup> This agent did not show efficacy as single agent, but proved to be effective in combination with both lenalidomide and bortezomib.<sup>19,20</sup>

Daratumumab is a human IgG1 mAb that targets CD38-expressing cells. Daratumumab binding to CD38 induces tumor cell death through direct and indirect mechanisms. It can induce apoptosis via CD38 cross-linking and have an anti-tumor effect mediated by the activation of complement dependent cytotoxicity, antibody dependent cell phagocytosis and antibody dependent cell cytotoxicity. CD38 is also expressed on highly immunosuppressive Treg and Breg cells, as well as on MDSC; daratumumab eliminates these highly immunosuppressive cells, thus stimulating cytotoxic T cell mediated antitumor effects.<sup>21–25</sup> Daratumumab showed efficacy as single agent in heavily pre-treated patients,<sup>26,27</sup> as well as in combination with bortezomib and with lenalidomide.<sup>28,29</sup>

Immunomodulatory drugs are of particular interest in combination with both mAbs and checkpoint inhibitors. In particular, lenalidomide enhances immunogenicity by inducing T-cell activation through increased tyrosine kinase activity of the CD28 receptor, downregulation of CD45RA on T cells, and downregulation of SOCS1 on stromal cells in the microenvironment.<sup>30–32</sup> The aim of vaccines is to increase the frequency of antigen-specific T cells or antibodies. Vaccination approaches in MM include idiotype vaccines, dendritic cell-based and GM-CSF-based vaccines and cancer testis antigens vaccines.

Nevertheless, the main limitations to the efficacy of this approach is the intrinsic immune dysfunction associated with the tumor itself, especially in the presence of high disease burden, and the use in a therapeutic setting and not prevention setting as for anti-pathogen vaccines.<sup>33</sup>

### **Checkpoint blockade therapy**

Inhibitory molecules known as “immune checkpoint proteins” are expressed by a variety of immune cells (e.g. T and NK cells) to control the intensity and duration of immune responses, playing a pivotal role in the

induction of peripheral self-tolerance and the limitation of tissue damage.<sup>34</sup> The avoidance of immune-mediated destruction is a well-known hallmark of cancer biology,<sup>35</sup> as discussed above. MM cells like many other cancer cells exploit this physiologic mechanism to escape the surveillance mediated by the immune system. The main immune checkpoint pathways targeted in MM are cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), programmed cell death protein 1 (PD1), programmed cell death ligand 1 (PD-L1) and Killer-cell immunoglobulin-like receptors (KIRs). Clinical results of their inhibition through mAbs in myeloma field are summarized in *Table 1*.

#### CTLA-4

CTLA-4 was the first immune checkpoint molecule fulfilling the requirements to be clinically targeted in cancer.<sup>36</sup> CTLA-4 is a fundamental negative regulator of T-cell function and its absence causes immune hyperactivation and lethal diffuse lymphocytic infiltration in mice models.<sup>37</sup> In T-cells, CTLA-4 antagonizes the activating signal mediated by CD28, competing for the same ligand (B7-1) expressed by dendritic cells.<sup>38</sup> Upon the binding of B7-1, CTLA-4 induces in T-cells antigen-specific anergy.<sup>39</sup>

In MM patients, CTLA-4 is significantly overexpressed on CD4+ and CD8+ T cells in the bone marrow but not in the peripheral blood, compared to healthy donors.<sup>2</sup> This evidence suggests an enhanced T-cell unresponsiveness towards MM cells, especially at the tumor site, that can be theoretically reverted by CTLA-4 inhibition. Building on this hypothesis and on the clinical success of CTLA-4 inhibition in melanoma patients,<sup>36</sup> two mAbs targeting CTLA-4 are in clinical development in MM.

Ipilimumab (IgG1, fully human) has been studied as a single agent in 29 hematologic patients (21% with MM) relapsing from allogeneic stem-cell transplantation in order to stimulate the *graft-versus-tumor* effect mediated by effector T-cells in this setting,<sup>40</sup> but no objective responses were elicited in MM patients. Due to the peculiar mechanism of action, the major risk associated with checkpoint blockade therapy is to induce autoimmune-like syndromes, known as immune-related adverse events (IRAEs). This aspect is very relevant in the allogeneic setting, with an additional risk of *graft-versus-host* disease (GvHD). In this trial, ipilimumab did not induce grade 3-4 GvHD or graft rejection. IRAEs were registered in 4 patients (1 grade 3 polyarthritis, 1 grade 2 hyperthyroidism, 1 recurrent grade 4 pneumonitis and 1 obstructive defect with no infectious cause identified), however none of them had MM.



Trials in MM patients are currently evaluating ipilimumab-based combinations (NCT01592370), comparing ipilimumab with another checkpoint inhibitor acting on a different target (nivolumab) in the allogeneic SCT setting (NCT01822509) and combining ipilimumab with nivolumab in the autologous SCT setting (NCT02681302).

Tremelimumab (IgG2, fully human) is another CTLA-4 targeting mAb. Although no clinical data are yet available in MM patients, a trial involving tremelimumab-combination therapy with another checkpoint inhibitor acting towards a different target (durvalumab) after autologous SCT is currently planned (NCT02716805).

### PD-1/PD-L1

The immune checkpoint pathway that produced the most promising preclinical and clinical data in MM is based on the interaction between two transmembrane proteins: PD1 and PD-L1.<sup>41</sup> PD-1, differently from CTLA-4, is expressed not only by T cells, but also by NK cells, B cells and other immune cell subsets, playing a pivotal role in the balance between immune system activation and self-tolerance.<sup>42</sup> Differently from CTLA-4, PD-1 deficient mice develop lupus-like systemic inflammation,<sup>43</sup> suggesting that CTLA-4 and PD-1/PD-L1 axis may work in slightly different aspects of immune regulation. *Table 2* summarizes the main similarities and differences in CTLA-4 and PD-1.

PD-L1 is expressed by antigen-presenting cells and other cell subtypes, delivering inhibitory signals through the engagement of PD-1.<sup>44</sup> Normal plasma cells do not express PD-L1, while PD-L1 is expressed on the surface of MM cell lines and neoplastic plasma cells isolated from MM patients.<sup>45,46</sup>

PD-1 may bind another ligand besides PD-L1, known as PD-L2. PDL2 expression could be detected in the microenvironment of solid tumors, however it does not seem to be a key inhibitor of T-cell function at the tumor site.<sup>47</sup>

There is evidence that blocking PD-1 and/or PD-L1 through mAbs in order to prevent their interaction can enhance myeloma-cell killing by T and NK-cells *in vitro*.<sup>48</sup> Moreover, in a MM mouse model, Hallett and colleagues demonstrated that PD-L1 on MM cells engaging PD-1 expressed by T-cells and NK cells, decrease their cytotoxic function, proliferation, and cytokine production, thus leading to a functionally exhausted state of these cells.<sup>46</sup>

Based on these biologic data and on the success of mAbs targeting PD-1 in solid tumors<sup>49</sup> and Hodgkin's lymphoma,<sup>50</sup> several mAbs targeting this pathway are being evaluated in MM.

Nivolumab is a fully human IgGk mAb targeting PD-1. A phase Ib trial evaluated its safety and efficacy as single agent in hematologic patients affected by several malignancies, among which 27 with relapsed and/or refractory MM (RRMM) patients. Unfortunately, in this subgroup of patients, no objective responses were reported.<sup>51</sup> Building on pharmacokinetic data demonstrating a long-lasting binding of Nivolumab to its receptor, Funt and colleagues recently reported on next line of therapy after nivolumab infusion in 8 RRMM patients.<sup>52</sup> An unusually low rate of progressive disease was reported in this highly refractory patient population and interestingly a patient who relapsed with an isolated plasmacytoma received only radiotherapy and then resumed Nivolumab treatment with a progression-free period of more than 2 years. Several ongoing trials are evaluating Nivolumab in combination with Daratumumab ± Pomalidomide-Dexamethasone (NCT01592370), Pomalidomide-Dexamethasone ± Elotuzumab (NCT02726581) and with anti-CTLA4 mAbs after autologous (NCT02681302) and allogeneic (NCT01822509) stem cell transplantation.

Pembrolizumab is a humanized anti-PD1 IgGk mAb. It is probably the anti-PD1 mAb evaluated in the greatest number of malignancy types. Data on monotherapy in MM field are lacking, however pembrolizumab has been tested in combination with dexamethasone and immunomodulatory agents in RRMM patients. A phase I study in 40 RRMM patients failing at least 2 previous therapeutic lines evaluated safety and efficacy of Pembrolizumab added to Lenalidomide-Dexamethasone backbone treatment.<sup>53</sup> The MTD for Pembrolizumab was 200 mg every 21 days and the overall response rate (ORR) was 50%. Of note, responses were also elicited in lenalidomide-refractory patients, suggesting that pembrolizumab and lenalidomide could act synergically also in patients refractory to immunomodulators alone. Safety profile was acceptable, and hematologic toxicities (thrombocytopenia 41%, neutropenia 37%) were the most common treatment-related adverse events. Of note, IRAEs were rare and the most frequent non hematologic toxicity was mild diarrhea (28%, all cases grade ≤ 2). A phase III randomized trial evaluating first-line treatment with lenalidomide-dexamethasone ± pembrolizumab in transplant ineligible MM patients (NCT02579863) is currently ongoing.

A phase II trial in 48 lenalidomide-refractory RRMM patients evaluated pembrolizumab in combination with pomalidomide and dexamethasone.<sup>54</sup> Besides lenalidomide, 80% of enrolled patients were also refractory to a proteasome inhibitor. The overall response rate (ORR) was 56% and responding patients had a median duration of response of more than 6 months. Interestingly, double-refractory patients had a superimposable ORR (55%). IRAEs reported in this trial were interstitial pneumonitis (13%), hypothyroidism (10%), hepatic cytolysis (6%), adrenal insufficiency (4%) and vitiligo (2%). Additional data will come from a phase III trial in RRMM patients evaluating pomalidomide-dexamethasone  $\pm$  pembrolizumab (NCT02576977) that is currently enrolling patients.

PD-1 inhibition in MM is in advanced clinical development, with 2 phase III studies underway. However, little is known about targeting its cognate receptor, PD-L1, and about the clinical differences between these approaches.<sup>55</sup> Durvalumab (MEDI-4736) and Atezolizumab (MPDL3280) are being used in phase I trials in MM patients in different settings and with different combinations (*Table 1*), however no data are available yet.

#### Killer-cell immunoglobulin-like receptor

Killer-cell immunoglobulin-like receptors (KIRs) are key regulators of NK cell cytotoxic function. Inhibitory and activating KIRs regulate NK cells through the recognition of major histocompatibility class I molecules on target cells.<sup>56</sup> NK cells harvested from MM patients express inhibitory KIRs,<sup>57</sup> moreover malignant plasma cells express their cognate surface ligands,<sup>58</sup> like major histocompatibility complex (MHC) class I and related proteins. Nijhof and colleagues demonstrated that blocking inhibitory KIRs on NK cells *in vitro* improves daratumumab-induced anti-myeloma activity and this effect was particularly evident when lenalidomide was added.<sup>59</sup>

Based on this rationale, IPH2101, a human IgG4 mAb targeting KIR2D receptors, has been evaluated in MM patients. In a phase I trial, 32 RRMM patients treated with IPH2101 monotherapy did not show any objective responses, however safety was very good with no dose limiting toxicities.<sup>57</sup> The lack of single-agent activity was confirmed also in the smoldering MM setting by a phase II trial.<sup>60</sup> A phase I trial evaluated the combination of IPH2101 with lenalidomide in RRMM patients.<sup>61</sup> Safety profile was acceptable and objective responses were reported in 5 out of 15 enrolled patients. Nevertheless, the small number of patients and the exclusion of lenalidomide-refractory patients make it difficult to draw conclusions on the

efficacy of IPH2101 in the context of immunomodulatory pre-treated patients.

Another mAb targeting KIRs (Lirilumab) is under development. Combinations study with elotuzumab (NCT02252263) and nivolumab (NCT01592370) are currently ongoing.

### **CAR-T based ACT**

CAR-T cells are autologous or allogeneic T cells genetically engineered to express a chimeric antigen receptor specific for a tumor-associated antigen expressed on the neoplastic cell surface. CARs are also "equipped" with co-stimulatory domains, which enhance activation and function of CAR-T cells, and promote their proliferation and cytokine release.<sup>62</sup> CAR-T cells have both advantages and limitations. They are not restricted by patient HLA. However, selecting appropriate antigens is crucial to prevent on-target off-tumor toxicity. Many potential targets have a broad expression on normal cells and tissues.<sup>63</sup> Major clinical trials have so far employed CAR-T cells that greatly differed in both targeted-antigens and co-stimulatory domains.

CAR-T cells directed against CD19 initially showed a dramatic potential in acute lymphoblastic leukemia and chronic lymphocytic leukemia.<sup>64-66</sup> Even though CD19 expression is not usually associated with MM and it is not considered a therapeutic target,<sup>67</sup> some studies identified its expression on a putative minor MM stem cell subset<sup>68</sup> that may partly be responsible for disease recurrence. Despite being hotly debated, the potential existence of a CD19+ MM stem cell formed the rationale for conducting clinical trials with specific CAR against CD19.<sup>69</sup> Garfall et al. initially described one patient treated with anti-CD19 CAR- T cells (CTL019).<sup>69</sup> The patient underwent CTL019 infusion after a standard autograft. Following cell infusion, no fever or other signs of cytokine release syndrome (CRS) were noted and, importantly, CTL019 cells were detected in both blood and bone marrow for up to several days after the infusion. The patient started lenalidomide maintenance 3 months later. Complete response (CR) was still observed at one year of follow-up. It is remarkable that the response was achieved despite the absence of CD19 expression on 99.95% of plasma cells. An update of this clinical trial on a series of 12 patients was reported at American Society of Hematology (ASH) meeting in December 2016.<sup>70</sup> Overall, 10 out of 12 patients enrolled were infused with CTL019 12–14 days after high-dose melphalan and an autograft. Six patients showed very good partial response (VGPR), 2 partial response (PR), 2 progressive disease (PD). Of note, only minor adverse effects were reported.

More recently, the B cell maturation antigen (BCMA) has drawn increasing attention as a CAR target in MM. BCMA is a cell surface protein involved in the differentiation and maturation of B cells into plasma-cells. However, BCMA is highly expressed also on MM cells. Ali et al reported on a phase I study with an anti-BCMA-CAR with a CD28 co-stimulatory molecule.<sup>71</sup> Twelve patients received escalating doses of CAR-T cells at 0.3, 1, 3, or  $9 \times 10^6$ /kg. All patients were immune-suppressed with cyclophosphamide and fludarabine prior to CAR-T cells infusions. Responses included stringent CR (n=1), VGPR (n=2), PR (n=1), and SD (n=8). Both best responses and side effects occurred in the highest-dose patients. Only one single patient has so far been reported to relapse in the marrow with BCMA-negative MM clones.<sup>71</sup> Two other BCMA specific CAR-T cells are currently being investigated in clinical trials. Preliminary findings were presented at the 2016 ASH meeting by the University of Pennsylvania group.<sup>72</sup> In a phase I dose-escalation study, with a second generation 4-1BB-CD3 $\zeta$  anti- BCMA-CAR-T cells, 6 patients received split dose infusions, 10% on day 0, 30% on day 1, and 60% on day 2. Overall, 5 patients developed CRS toxicity; 2 patients required anti IL-6 treatment with tocilizumab. Interestingly, even though these 2 patients had received only 40% of the planned CAR-T cell dose, they achieved a remarkable anti-tumor response with a stringent CR and a VGPR, respectively. Five months after the last infusion, the patient who achieved VGPR progressed with a concomitant reduction of circulating CAR-T cells. Of note, BCMA expression was lost on MM cells, which is highly suggestive of antigen escape. Overall, the 4 other patients showed stable disease, minimal response, or PD with low expansion of CAR-T cells. Bluebird Bio reported on a cohort of 9 patients with RRMM who were infused with second-generation anti-BCMA-CAR-T cells (bb2121) with 4-1BB co-stimulation.<sup>73</sup> Patients received single infusions at different doses (5, 15, or  $45 \times 10^7$ ) after being conditioned with cyclophosphamide and fludarabine. Best responses were achieved after the infusion of  $15 \times 10^7$  CAR T cells: 2 patients showed stringent CR and 1 VGPR.

CD138 (also known as syndecan 1) is a surface protein expressed on normal and malignant plasma cells,<sup>74</sup> and it binds collagen and fibronectin molecules in the extracellular matrix. Given its high expression on MM cells, it has been considered an attractive target. A Chinese group reported their experience with an anti CD138 CAR on 5 refractory patients.<sup>75</sup> Multiple infusions were well tolerated and only mild fever was reported, stable disease was achieved in 4 out of 5 patients. Nevertheless, these are preliminary findings and

further confirmation is awaited. Importantly, CD138 is not specifically expressed by MM cells but it is also expressed by epithelial cells raising some concerns regarding on-target off-tumor toxicity. Other clinical and preclinical studies with CAR-T cells directed against other MM specific targets are in progress. A phase I clinical trial of kappa-CD28-CAR-T cells was also conducted at Baylor hospital.<sup>76</sup> Two other studies with anti CS-1 CAR-T cells (NCT02203825) and anti CD138-CAR-T cells (NCT01886976) are currently recruiting patients, and results are eagerly awaited.

Overall, CAR-T cells are a promising form of immune-therapy in MM. Nonetheless, some relevant concerns exist. Antigenic escape is a major limitation of CAR-T cells targeted against a single antigen on MM cells. To overcome this phenomenon, targeting two different antigens may improve specificity and efficacy of CAR-T cells. Targeted antigens expressed by T-cells themselves and leading to self-killing of CAR-T cells are another important issue.<sup>77</sup> Other concerns have been raised regarding efficacy, such as depth and duration of antitumor effects, and toxicity profile, such as the potential serious systemic toxicities including CRS.<sup>78</sup> Moreover, high tumor burdens may be associated with higher risk of CRS. Anecdotal specific toxicities have also been reported. The Pennsylvania group recently published a case report of posterior reversible encephalopathy syndrome (PRES) after infusion of anti-BCMA CAR-T cells with worsening neurological symptoms despite treatment with tocilizumab and high-dose steroids. However, cyclophosphamide administration reversed the syndrome.<sup>79</sup>

## **Conclusion**

Advances in the understanding of MM biology and its clinical management have recently led to an increased survival rate, reaching up to 8-10 years. Several new agents, with different mechanism of actions and different targets, have increased the treatment armamentarium against a complex disease such as MM. These agents include chemotherapeutic agents, immunomodulatory drugs and proteasome inhibitors, which are currently considered the backbone treatments for MM. Other agents more recently introduced include histone deacetylase inhibitors, the so called “small molecules”, monoclonal antibodies, checkpoint inhibitors and ACT. Acting on immune system is an appealing new treatment strategy, where checkpoint inhibitors and ACT are the main players. Results on checkpoint blockade in MM are promising, but not yet as good as in solid tumors. One of the reasons could be the impaired immune function that characterizes immune system

of MM patients. The most promising results have been so far achieved in MM in combination with immunomodulatory drugs, due to the potential synergistic effect on the immune system. Furthermore, checkpoint molecules that are currently clinically exploited are only the tip of the iceberg of the potential checkpoint targets expressed by immune-cells. In the near future, data on mAbs targeting lymphocyte activation gene 3 protein (LAG-3), T cell immunoglobulin domain and mucin domain 3 (TIM-3), V-domain Ig suppressor of T cell activation (VISTA), CD47 and other molecules might be available to researchers. The preliminary clinical success of CAR-T cells in lymphoblastic leukemia has reignited the interest in cell immunotherapy against MM and this field is now moving forward very rapidly. However, despite durable responses in heavily pretreated patients, results are very preliminary and many open questions remain, including the definition of the best target, a better understanding of toxicities (e.g. CRS) and efficacy (depth of response and its duration). Multiple therapies are currently available for MM and many, new approaches will probably be available in the next future.

Future and ongoing trials are still necessary to shed light on the role of checkpoint inhibitors and CAR-T cells in a treatment scenario in continuous evolution.

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**Table 1.** Clinical data from key studies exploring mAb-based treatment in RRMM patients

TARGET	DRUG	COMBINATION	STUDY PHASE	PATIENT POPULATION (number of MM patients)	CLINICAL RESULTS AVAILABLE Y/N	ORR (%)	Reference or NCT
CTLA-4	Ipilimumab	-	1	Relapsed hematologic malignancies after allogeneic SCT (6)	Y	0 <sup>1</sup>	Bashey et al <sup>40</sup>
		Ipilimumab monotherapy vs Nivolumab monotherapy	1	Relapsed hematologic malignancies after allogeneic SCT (na)	N	-	NCT01822509
		Ipilimumab+nivolumab	1b/2a	Lymphoma and MM patients at high risk of relapse after ASCT (na)	N	-	NCT02681302
	Tremelimumab	Durvalumab	1	TE MM patients before and after ASCT (24)	N	-	NCT02716805
PD-1	Nivolumab	-	1b	RR hematologic malignancies (27)	Y	0 <sup>1</sup>	Lesokhin et al <sup>51</sup>
		Arm 1: nivolumab monotherapy Arm 2: Nivolumab + Ipilimumab / Lirilumab Arm 3: Daratumumab + Nivolumab ± Pomalidomide- Dexamethasone	1	RRMM ≥ 2 prior therapies (375*)	N	-	NCT01592370
		Arm 1: nivolumab + Pomalidomide- Dexamethasone Arm 2: Pomalidomide- Dexamethasone Arm 3: nivolumab + Elotuzumab + Pomalidomide- Dexamethasone	3	RRMM ≥ 2 prior therapies (406*)	N	-	NCT02726581
	Pembrolizumab	-	1	Smoldering MM (16)	N	-	NCT02603887
		Lenalidomide-dexamethasone	1	RRMM ≥ 2 prior therapies (40)	Y	50%	Mateos et al <sup>53</sup>
		Lenalidomide-dexamethasone ± Pembrolizumab	3	NTE NDMM (640*)	N	-	NCT02579863

		Pomalidomide-dexamethasone	1/2	Lenalidomide-refractory RRMM $\geq 2$ prior therapies (48)	Y	65%	Badros et al <sup>54</sup>
		Pomalidomide-dexamethasone $\pm$ Pembrolizumab	3	RRMM $\geq 2$ prior therapies (300*)	N	-	NCT02576977
PD-L1	Durvalumab	Arm A: durvalumab + lenalidomide- dexamethasone in high risk NTE NDMM Arm B: durvalumab + lenalidomide- dexamethasone in standard risk NTE NDMM Arm C: durvalumab +lenalidomide maintenance for high risk post-ASCT NDMM	1b	NDMM (138*)	N	-	NCT02685826
		Arm A: durvalumab monotherapy Arm B: durvalumab + pomalidomide Arm C: durvalumab + pomalidomide- dexamethasone	1b	RRMM $\geq 2$ prior therapies (138*)	N	-	NCT02616640
	Atezolizumab	-	1	Solid and hematologic tumors (na)	N	-	NCT01375842
		Arm A: monotherapy Arm B: atezolizumab-lenalidomide Arm C: atezolizumab post-ASCT Arm D: atezolizumab + daratumumab Arm E: atezolizumab + daratumumab + lenalidomide Arm F: atezolizumab + daratumumab + pomalidomide	1	Arm A-E: RRMM $\geq 1$ but $\leq 3$ prior therapies  Arm F: RRMM $\geq 3$ prior therapies  (214*)	N	-	NCT02431208
KIRs	IPH2101	-	1	RRMM $\geq 1$ prior therapies (32)	Y	0	Benson et al <sup>80</sup>
		-	2	Smoldering MM (9)	Y	0	Korde et al <sup>60</sup>
		Lenalidomide	1	RRMM 1 or 2 prior therapies (15)	Y	33%	Benson et al <sup>81</sup>
	Lirilumab	Arm A: Lirilumab + Elotuzumab Arm B: Urelumab + Lirilumab	1	Post-ASCT RRMM achieving at least a very	N	-	NCT02252263

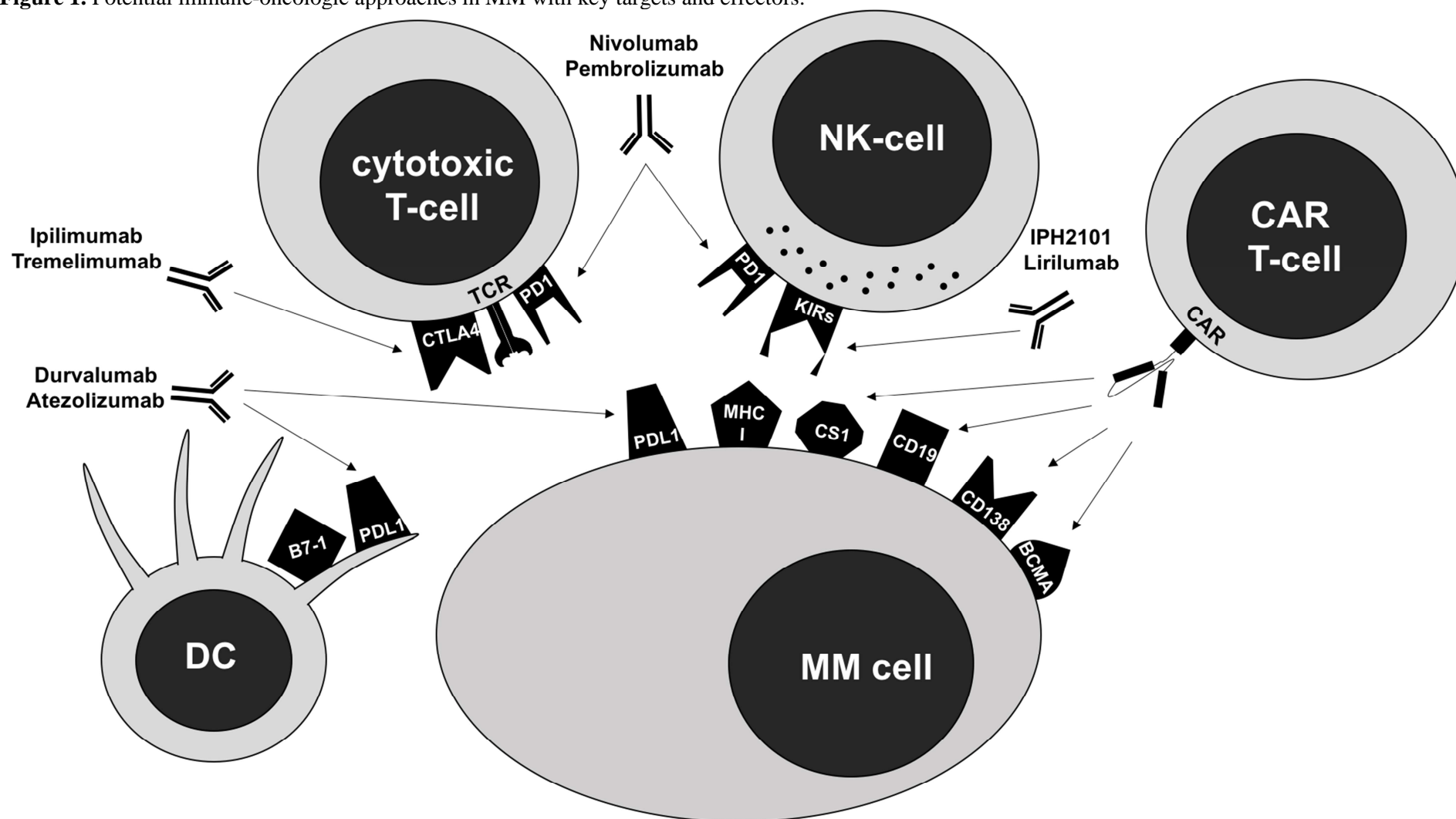




**Table 2.** Similarities and differences between CTLA-4 and PD1 molecules.<sup>37,43,82,83</sup>

CTLA4	PD1
Knock-out mouse: lymphoproliferative phenotype predominant	Knock-out mouse: autoimmune phenotype predominant
Expressed by T-Cells	Expressed by many immune cell subsets
Reduce T-cell proliferation, cytokine production and effector function.	Reduce T-cell proliferation, cytokine production and effector function.
Expression level on T cells affected by intensity and duration of TCR signaling	Expression level on T cells affected by intensity and duration of TCR signaling
Ligand CD28 expressed by professional immune cells	Ligand PD-L1 expressed by immune and nonimmune cells (e.g. endothelium, tumor cells)
No data on CD28 expression and response correlation	PD-L1 expression on tumor cells may predict clinical responses

**Figure 1.** Potential immune-oncologic approaches in MM with key targets and effectors.



**Legend.** TCR: t-cell receptor; CAR: chimeric antigen receptor; PD1: programmed cell death protein1; PDL1: programmed cell death ligand 1; CTLA4: cytotoxic T-lymphocyte-associated antigen 4; KIRs: Killer-cell immunoglobulin-like receptors; MHC I: histocompatibility class I molecules; CS1: Signaling lymphocytic activation molecule F7 or SLAMF7; BCMA: B-cell maturation antigen; B7.1: Activation B7-1 Antigen or CD80.